USDA ARS National Animal Germplasm Program

Poultry Gonad Collection, Processing and Vitrification Protocol

All methods were developed by scientists at Agriculture and Agri-Food Canada and approved by the Pacific Agri-Food Research Center (Agassiz) Animal Care Committee. The protocol follows principles outlined by the Canadian Council of Animal Care (reformatted 2011). http://www.ccac.ca/en_/standards/guidelines

Policy:

Removal of ovaries and testes for cryopreservation in chicken should optimally be performed within 24 hours of hatch. In chicks older than 1 day of age the transplantation of gonadal tissue becomes entirely dependent on the use of an immunosuppressant.

Procedure:

Gonad collection:

Sacrifice chicks by cervical dislocation and open the abdominal cavity to expose the gonads.

NOTE: In chicks, the testes are paired organs located in the dorsal part of the abdomen on either side of the median plane, and the single ovary is an irregularly shaped structure attached to the dorsal abdominal wall on the left side of the cavity.

Detach gonads with fine forceps and/or fine scissors.

Connective tissue attached to the gonads may be removed at this time, or following vitrification and thawing, with the aid of a dissecting microscope.

Cut the end of each testicle to allow the media (cryoprotective agents) to penetrate the tissue.

NOTE: If necessary, right and left testicles may be processed and vitrified and stored on separate acupuncture needles to ensure that individuals will not be represented more than once when material is recovered.

Keep gonads on ice in approximately 5.0 mL of Holding Medium prior to vitrification. Hold samples in this state no longer than 4 h.

Cut ovaries in half and place the two halves on one acupuncture needle. Do likewise with a second ovary.

Place whole testes on acupuncture needles; 4 testes per needle.

Vitrification:

Prepare a box containing liquid nitrogen, 50 mL tubes containing liquid nitrogen, a metal platform within the liquid nitrogen so that the level of the LN2 is at the top of the platform, and a rack to hold labeled 5 mL cryovials positioned upon the metal platform. Allow this to cool until the liquid nitrogen stops boiling.

Place the samples/needles holding gonads in the Equilibration Solution for 10 minutes ensuring that the tissue is submerged in the solution.

Transfer the needles to Vitrification Solution for 2 minutes.

Blot the gonadal tissue on gauze to remove excess solution and immediately plunge the samples into LN2.

After the samples stop boiling in the liquid nitrogen, needles are placed in a 5 mL pre-cooled cryovial that is positioned in a rack in the liquid nitrogen vapor.

Seal the cryovials with tops containing gaskets and store in liquid nitrogen.

Thawing:

Samples are removed from liquid nitrogen, immersed in Holding Medium supplemented with 1 M sucrose at 40 °C for 5 min and then subsequently transferred to 0.5, 0.25, and 0 M Holding Medium-sucrose solutions for 5 min each at room temperature.

Tissue samples can be maintained in Holding Medium on ice prior to transplantation.

MATERIALS:

Ethylene Glycol (EG)
Dimethylsulfoxide (DMSO)
Dulbecco's phosphate buffered saline (DPBS)
Fetal Bovine Serum (FBS)
Sucrose
Sterilized sharp surgical scissors, scalpel blade, fine forceps (Dumont),
Sterile gauze
Petri dish
Ice
Liquid nitrogen
Styrofoam box
5 mL cryotubes with a small hole in the top
50 mL tubes
Acupuncture needles
Dissecting microscope

Recipes:

Holding Medium:

Dulbecco's phosphate buffered saline (DPBS) with 20% Fetal Bovine Serum (FBS)

Equilibrium Solution:

85 % Holding Medium
7.5 % Ethylene glycol (EG)
7.5 % Dimethylsulfoxide (DMSO)
Vitrification Solution:

15 % EG
15 % DMSO
0.5 M sucrose
Holding Solution to final volume

References:

J. Liu, K.M. Cheng, P.H. Purdy, F.G. Silversides. 2012. A simple vitrification method for cryobanking avian testicular. Poultry Science. 91: 3209-3213. https://doi.org/10.3382/ps.2012-02454.

F.G. Silversides, M.C. Robertson, J. Liu. 2013. Cryoconservation of avian gonads in Canada. Poultry Science. 92: 2613-2617. https://doi.org/10.3382/ps.2013-03185.

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